

Photoelectric Light-Scattering Photometer for Determining High Molecular Weights*

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A photoelectric photometer designed for the measurement of absolute turbidity, dissymmetry, and depolarization of dilute solutions of high molecular weight materials, and hence determination of their molecular weights, is described. The photometer comprises essentially a monochromatic parallel primary beam of radiation, a six-sided scattering cell for measurements at 0°, 45°, 90°, and 135°, a multiplier photo-tube and galvanometer, a standard opal glass diffusor, and removable polarizer and analyzer. Turbidity is determined in terms of a ratio of deflections for the 90° scattering and for the primary beam reduced in intensity by neutral filters and diffused by an opal glass plate. Working relationships leading to determination of absolute turbidity are developed. These relationships include corrections for refraction and reflection effects, and for imperfect diffusion by the opal glass. The latter is evaluated by comparison of the opal glass with reflecting diffusors corrected for specular component of reflectance. The response of some multiplier photo-tubes is shown to be dependent on the plane of polarization of the incident radiation. Data illustrating the performance of the photometer include comparison of molecular weights of polystyrene fractions, beta-lactoglobulin, bovine serum albumin, lysozyme, sucrose octaacetate, Raleigh's ratio and depolarization for benzene, turbidity of a "standard" polystyrene, and particle size of a GR-S latex, with data obtained by other methods or other investigators.

I. INTRODUCTION

THE theory of scattering of light by solutions was presented by Debye^{1,2} in a form which permits determination of the molecular weight of a solute from readily measurable optical quantities, the turbidity and the specific refractive increment of its solutions. The speed and relative simplicity of the method, as well as the additional information which it gives regarding particle size and anisotropy, have established it as a useful tool in studies of polymers. A number of papers have

presented the theory and some of its applications in more detail.³

Debye showed that the reciprocal specific turbidity, c/τ , is related to the weight-average molecular weight, M , of the solute in accordance with the following expression, provided the solute molecules are isotropic and small as compared with the wave-length of the light used:

$$Hc/\tau = (1/M) + (2B/RT)c + \dots \quad (1)$$

$$H = \frac{32\pi^2 n_0^2 (n - n_0)^2 / c^2}{3\lambda^4 N_0} \quad (2)$$

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¹ P. Debye, J. App. Phys. 15, 338 (1944).

² P. Debye, J. Phys. and Colloid Chem. 51, 18 (1947).

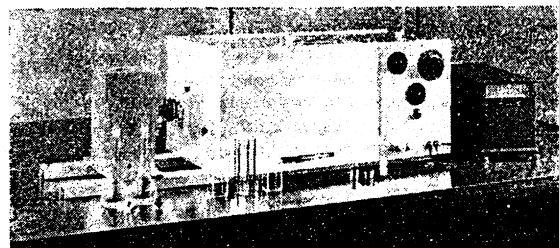
³ See, for example, B. H. Zimm, R. S. Stein, and P. Doty, Polymer Bull. 1, 90 (1945); G. Oster, Chem. Rev. 43, 319 (1948); R. H. Ewart, C. P. Roe, P. Debye, and J. R. McCartney, J. Chem. Phys. 14, 686 (1946); P. M. Doty, B. H. Zimm, and H. Mark, J. Chem. Phys. 13, 159 (1946).

where τ is the turbidity [defined by $P = P_0 e^{-\tau x}$, see Eq. (10)] of the solution minus that of the solvent, c is the concentration in grams per milliliter of solution, B is a constant depending on the solvent, R is the gas constant, T is the absolute temperature, n and n_0 are the refractive indices of solution and solvent, λ is the wave-length in vacuum of the primary radiation, and N_0 is Avogadro's number.

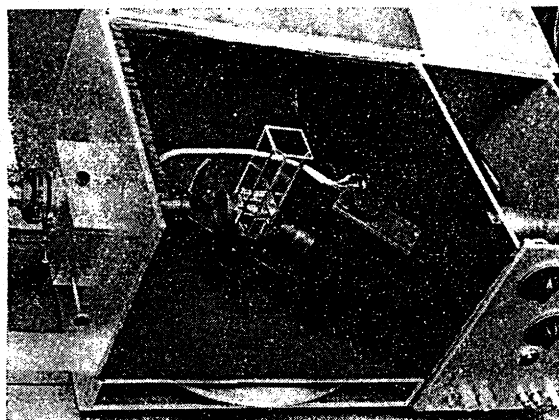
If Hc/τ is determined for a number of different concentrations of polymer in solution and plotted against c , a straight line should result. The intercept, or the value of Hc/τ for infinite dilution, should be the reciprocal of the weight-average molecular weight.

The turbidity τ is usually evaluated from scattering measurements at 90° to the primary beam. If the transversely scattered light due to the solute shows appreciable depolarization caused by anisotropy or large size of the solute particles, or if the scattered light shows dissymmetry (greater forward than backward scattering) due to particle dimensions greater than about $\lambda/10$, appropriate corrections¹⁻³ must be applied to the observed turbidities.

The determination of relative turbidities by measurement of transverse scattering is not difficult; however, the evaluation of absolute turbidity from such measure-



A



B

FIG. 1. Photometer. A shows lamp housing; light-proof Duralumin box, $24 \times 16 \times 11$ in., containing the scattering system, receiver, and power supply; galvanometer, scattering cells; removable polarizer and analyzer; and opal glass standard diffusor; B (lids open and front panel removed) shows receiver set at 45° to primary beam, graduated disk, dissymmetry cell, and working standard.

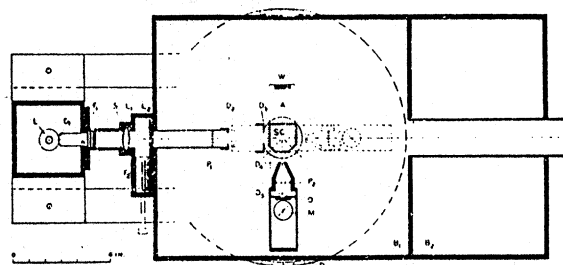


FIG. 2. Diagrammatic sketch of optical system: L , mercury lamp; F_1 , monochromatic filters; S , camera shutter; F_2 , neutral filters mounted on a sliding carriage; L_1 , achromatic lens ($f = 122$ mm); L_2 , cylindrical lens ($f = 200$ mm) with axis horizontal; SC , scattering cell (40×40 mm) on fixed table; D , graduated disk attached to rotatable arm, A , carrying removable working standard, W , and the receiving system (shown in 90° position, 0° position dotted); limiting rectangular diaphragms in primary beam, D_1 (13 mm wide \times 11 mm high), D_2 (15 \times 15 mm), and D_3 (12 \times 15 mm); limiting rectangular diaphragms in receiving system; D_4 (3.1 \times 6.4 mm) and D_5 (7.4 \times 22 mm); O , opal glass depolarizing diffusor close to the multiplier photo-tube, M ; P_1 and P_2 , positions of demountable polarizer and analyzer; T , blackened removable tube serving as a light trap and as a means of aligning parts; H , covered peephole; B_1 , scattering compartment; B_2 , power supply compartment.

ments is beset with many difficulties, which require careful attention to geometrical design, application of certain corrections, and elimination of many sources of error. The only instruments heretofore established for the measurement of absolute turbidities of solutions for molecular weight determination are the absolute turbidity camera^{2,4,5} developed in Professor Debye's laboratory and a photoelectric apparatus developed in Zimm's laboratory.⁶ No complete data illustrating the performance of these instruments have been published. The visual turbidimeter described by Stein and Doty⁷ would appear to be an absolute instrument. The reported close agreement between molecular weights determined on this instrument and by osmotic pressure may be fortuitous, however, since corrections for refraction and other effects were not considered. A number of photoelectric instruments calibrated with substances of known turbidity or known scattering power have recently been described: that of P. P. Debye,⁸ calibrated with solutions measured on the absolute camera of Billmeyer and Debye; those of Stamm *et al.*,⁹ and Zimm,¹⁰ both calibrated with benzene; that of Blaker *et al.*,¹¹ calibrated with carbon disulfide; and

⁴ F. W. Billmeyer, Jr., Rubber Reserve Company, Technical Report, "The absolute determination of scattered intensity" (January 15, 1945).

⁵ F. W. Billmeyer, Jr., Rubber Reserve Company, Technical Report, "The determination of molecular weights and particle sizes by light scattering" (March 1, 1945).

⁶ C. I. Carr, dissertation, University of California, "An investigation of the molecular weights of molecules in solution by the light scattering method" (September, 1949). See also P. Outer, C. I. Carr, and B. H. Zimm, *J. Chem. Phys.* 18, 830 (1950).

⁷ R. S. Stein and P. Doty, *J. Am. Chem. Soc.* 68, 159 (1946).

⁸ P. P. Debye, *J. App. Phys.* 17, 392 (1946).

⁹ R. F. Stamm, T. Mariner, and J. K. Dixon, *J. Chem. Phys.* 16, 423 (1948).

¹⁰ B. H. Zimm, *J. Chem. Phys.* 16, 1099 (1948).

¹¹ R. H. Blaker, R. M. Badger, and T. S. Gilman, *J. Phys. and Colloid Chem.* 53, 794 (1949).

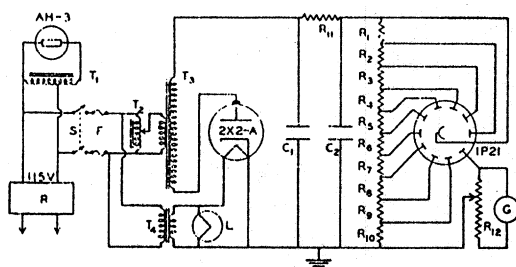


FIG. 3. Diagram of electrical circuit. *R*, electronic voltage regulator; *AH-3*, mercury lamp with autotransformer, *T*₁; *S*, switch, d.p.s.t.; *F*, fuses, 2a, 250 v; *T*₂, variable transformer; *T*₃, transformer, 800 v, r.m.s.; *T*₄, transformer, 2.5 v, 5a; *L*, pilot lamp; 2X2-A, rectifier tube; *C*₁ and *C*₂, condensers, 1 μ f, 1500 v; *R*₁–*R*₁₀, fixed resistors, 125,000 ohms (matched ± 2 percent), $\frac{1}{2}$ watt; *R*₁₁, 5000 ohms, 2 watts; 1P21, multiplier photo-tube; *G*, galvanometer; *R*₁₂, galvanometer shunt, 15,000 ohms.

that of Hadow *et al.*,¹² calibrated with benzene and with a polystyrene fraction of known molecular weight. Among instruments for measuring relative turbidity should be mentioned the photoelectric photometer of Putzeys and Brosteaux,¹³ who showed that the relative scattering powers of proteins at infinite dilution were proportional to their molecular weights.

It is the purpose of the present paper to describe and illustrate the performance of a photoelectric light-scattering photometer for the determination of absolute turbidity, dissymmetry, and depolarization of dilute solutions of high molecular weight compounds. An earlier model¹⁴ of this instrument yielded molecular weights of about half the accepted values, and an intensive study of the factors involved was necessary before the final instrument was established as an absolute turbidimeter. It is believed that the details of design and performance presented here will be of value to the many laboratories using or constructing equipment of this sort, particularly since previous publications have been somewhat lacking in experimental details.

II. OPTICAL AND ELECTRICAL SYSTEM

Figures 1 and 2 show the general design of the photometer. The light source is a high pressure Type *AH-3* mercury lamp.¹⁵ The monochromatic filters, for isolating wave-lengths 436 and 546 $m\mu$, are combinations of Corning glasses: blue filter, No. 3389 plus No. 5113 (2.5 mm), transmittance 20 percent at 436 $m\mu$, and less than 0.5 percent at 418 and 488 $m\mu$; green filter, No. 3484 plus No. 5120 (5 mm) plus No. 4305 (4.3 mm), transmittance 19 percent at 546 $m\mu$, and less than 0.5 percent at 525 and 576 $m\mu$. Neutral filters of transmittance approximately 50, 25, 12, and 6 percent are

required for reducing primary beam flux by known fractions during the measurements.

By means of the lens and diaphragm system shown, an approximately parallel, but slightly diverging, beam of radiation passes through the scattering cell, *SC*, and is absorbed in the light trap, *T*. The cylindrical lens, *L*₂, reduces the divergence in the vertical direction. The scattering cell is a sinter-fused optical glass cell so constructed that the cell contents can be viewed normally through windows at 0°, 45°, 90°, and 135° to the direction of the transmitted beam.

The polarizer and analyzer are Polaroid sheets mounted in removable tubes, and located in positions *P*₁ and *P*₂ when in use. Handles attached to the tubes move in accurately milled slots so that the electric vector can be made vertical or horizontal for depolarization measurements. Final alignment of the polarizing elements was made by use of a Nicol prism mounted at the peephole, *H*. The working standard, *W*, is a piece of flashed opal glass combined with a neutral filter of transmittance approximately six percent. As shown in Fig. 2, it moves into the primary beam when the receiver is set at 0°. The position of the receiver is controlled and indicated over a range $\pm 135^\circ$ by means of the projecting disk, *D*, graduated in degrees.

Figure 3 shows the electrical circuit. A Sorensen§ Model 250 electronic regulator furnishes stabilized voltage (115 ± 0.2 v) to the lamp transformer and to the power supply of a Type 1P21 multiplier photo-tube. Adjustment of multiplier sensitivity is provided by a Type 200-B Variac in the primary of the high voltage transformer. Output currents are indicated by a galvanometer of sensitivity approximately 0.001 μ a/mm. Measurements are made by a ratio of deflections method. The galvanometer scale is adjusted mechanically to read zero for the dark current, and nearly full-scale deflections are used wherever possible by suitable choice of sensitivity setting for scattering deflections and choice of neutral filters for primary beam deflections.

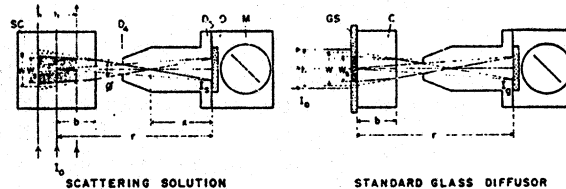


FIG. 4. Geometrical relationships (horizontal section) for scattering solution viewed at 90° and standard diffusor viewed at 0°. *SC*, scattering cell; *D*₁ and *D*₂, receiver diaphragms; *O*, diffusing opal glass near photo-tube *M*; *GS*, standard opal glass diffusor; *C*, compensating cell. In the apparatus (dimensions in mm), *b* = 22, *h* = 12, *L* = 15, *W* = 10, *r* = 89 (102 when opal glass, *O*, is removed). The angle $\phi/2 = 5.7^\circ$.

§ Mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

¹² H. J. Hadow, H. Sheffer, and J. C. Hyde, Can. J. Research 27B, 791 (1949).

¹³ P. Putzeys and J. Brosteaux, Trans. Faraday Soc. 31, 1314 (1935).

¹⁴ R. Speiser and B. A. Brice, J. Opt. Soc. Am. 36, 364A (1946).

¹⁵ B. T. Barnes and W. E. Forsythe, J. Opt. Soc. Am. 27, 83 (1937).

III. DETERMINATION OF DISSYMMETRY, DEPOLARIZATION, AND TURBIDITY

Dissymmetry, depolarization, and turbidity can be determined with the same cell. About 45 ml of solution are required. The cell must be carefully cleaned inside and out with a mild detergent. Pressure filtration of solutions through an ultrafine sintered-glass filter into the cleaned cell was found satisfactory for most solutions. The forward scattering of particles in the filled cell is examined by a low power microscope to determine whether filtration was adequate.

Dissymmetry is determined by a ratio of corrected galvanometer deflections for 45° and 135° positions of the receiver with a dilute solution in the cell. The sensitivity is adjusted to give a nearly full-scale deflection for the 45° position. Deflections observed for filtered solvent at these angles and with the same sensitivity setting are subtracted from the deflections observed for the solution. Dissymmetry coefficient is then

$$q = (G_{45}/G_{135}) - 1, \quad (3)$$

where G_{45} and G_{135} are the corrected deflections. In some cases it is necessary to determine q as a function of concentration and extrapolate to infinite dilution.

The *depolarization* for unpolarized incident radiation, ρ_u , is determined by inserting the analyzer, setting the receiver at 90°, reading a deflection, H_u , with the analyzer set to pass horizontal electric vector vibrations, and a deflection, V_u , with the analyzer set for vertical vibrations and with a neutral filter of relatively low transmittance, F , in the primary beam. Then

$$\rho_u = FH_u/V_u. \quad (4)$$

The depolarization for polarized incident radiation, ρ_v , is determined in a similar fashion, except that the polarizer is inserted and turned to pass vertical electric vector vibrations.

$$\rho_v = FH_v/V_v. \quad (5)$$

In both cases, deflections observed with the solvent under the same conditions must be subtracted from deflections observed with the solution before depolarization is calculated.

Turbidity is determined either by a "substitution" method or a "working standard" method. In the substitution method, the working standard (W , Fig. 2) is removed and a ratio of two nearly full-scale deflections is determined: one, G_s , observed for the scattering solution at 90°, and the other, G_o , observed at 0° (dotted position, Fig. 2) after the scattering cell is replaced by an opal glass standard diffusor. The deflection G_o is made roughly equal to the deflection G_s by inserting a neutral filter or combination of neutral filters of accurately known transmittance, F , in the

|| The standard diffusor is a sheet of solid opal glass, about 40×34×2.7 mm, with one side finely ground. It is mounted in a holder which locates the ground surface at the center of the sample table and faces it toward the receiver.

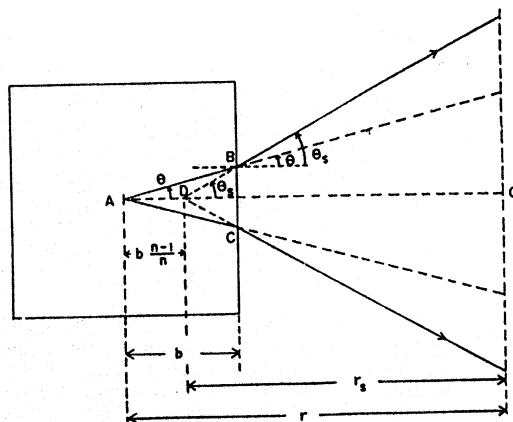


FIG. 5. Refraction effects: foreshortening of radius r to r_s , and spreading of solid angle from $\pi\theta^2$ to $\pi\theta_s^2$.

primary beam. Turbidity is proportional to G_s/G_o ; the final equation is shown later.

In the working standard method, a ratio of two nearly full-scale deflections is determined: one, G_s , is observed as before for the scattering solution at 90°; the other, G_w , is observed at 0° with the working standard in place (dotted position, Fig. 2), that is, the scattering cell is viewed by transmission. The deflection G_w is made roughly equal to the deflection G_s by the use of appropriate neutral filter or filters in the primary beam. The turbidity is proportional to G_s/G_w ; the final equation is shown later. This method is preferable to the substitution method, since the scattering compartment need not be opened between observations of the two deflections.

IV. DERIVATION OF TURBIDITY EQUATIONS

Figure 4 illustrates the geometrical relationships for the substitution method. In the diagram applying to the scattering solution, a parallel rectangular monochromatic beam of radiation of irradiance¹⁶ I_0 passes through the scattering cell, SC, of thickness $2b$. The width h and height L of the beam are determined by the dimensions of the diaphragm D_3 (Fig. 2). The receiving system views a radiating source which in a medium of unit refractive index would have dimensions W in width at its center, L in height, and h in depth; a volume $V = WLh$; and a center located at a distance r from the photo-tube diffusor, O . The width W is determined by the dimensions and locations of the diaphragms D_4 and D_6 , which were chosen so that $W < h$ and so that the receiver views a height greater than L .

Actually, however, refraction occurs when the scattered radiation passes from a solution of refractive index n to the receiving system in air. The effects of this refraction are as follows.

(a) *Shortening of W* (Fig. 4). The width W is reduced from W to W_s , resulting in a decreased irradiance at O .

¹⁶ Radiation terms and symbols are essentially those proposed by the Committee on Colorimetry of the Optical Society of America, J. Opt. Soc. Am. 34, 245 (1944).

By tracing the limiting rays:

$$\frac{W_s^*}{W'} = 1 - \frac{b}{r-x} \frac{n-1}{n}. \quad (6)$$

This ratio does not, however, represent the reduced irradiance at O , because elements of the radiating volume near its sides contribute less to the irradiance at O than do similar elements near the center of the volume. This refraction effect is therefore small; moreover, as will be shown, it can be compensated. In a similar way the height L is reduced to L_s , but this does not change the irradiance at O because the receiver views a height greater than L .

(b) *Foreshortening of r* (Fig. 5). The radiating volume center is displaced toward the receiver (A to D), resulting in an increased irradiance at O . By application of Snell's law to the refraction of rays AB and AC (the plane angle θ is small), $\theta_s = n\theta$, the displacement AD is $b(n-1)/n$ and the foreshortened radius is:

$$r_s = r \left(1 - \frac{b}{r} \frac{n-1}{n} \right). \quad (7)$$

(c) *Spreading of solid angle* (Fig. 5). Scattered flux within a small solid angle $\omega = \pi\theta^2$, directed normally toward the plane cell face, is spread into a solid angle $\omega_s = \pi\theta_s^2 = n^2\omega$ after refraction, resulting in a greatly decreased irradiance at O .

The irradiance at O for no refraction and for the actual refraction are in inverse ratio to the areas of caps of spheres having radii r_s and r :

$$\frac{I}{I_s} = \frac{\pi r_s^2 \theta_s^2}{\pi r^2 \theta^2} = \left(1 - \frac{b}{r} \frac{n-1}{n} \right)^2 n^2. \quad (8)$$

This represents the combined effects (b) and (c), and is in agreement with the expression derived independently by Carr⁶ for the case of a rectangular cell.¹⁷ If b

is small as compared with r , the ratio reduces to n^2 , the value used by Billmeyer.^{4,5} If $b=r$ (photo-tube cathode in contact with scattering cell or immersed in scattering medium), the ratio is unity, indicating no refraction effect.

In the diagram applying to the standard glass diffusor (Fig. 4), the same beam of irradiance, I_0 , is incident normally on the standard diffusor located in the center of the table formerly occupied by the scattering cell. To compensate for refraction effects (a) and (b), the diffusor is viewed through a compensating cell, C , of depth b filled with the scattering solution. The receiving system, set at 0° , thus views a diffusely irradiated area, $W_s L$, under a geometry similar to that for the scattering solution, the dimensions W_s , L , and r_s being identical in the two cases.

An equation for turbidity will now be derived from expressions relating the irradiances I_s and I_0 on the photo-tube diffusor to the incident irradiance I_0 . An expression for the total flux P (erg sec.⁻¹) emitted by a small volume in the scattering solution is obtained by integrating the irradiance (erg sec.⁻¹ cm.⁻²) over the surface of a sphere of radius r , or by integrating the quantity J_θ/r^2 over the surface, where J_θ is the radiant intensity (erg sec.⁻¹ ω.⁻¹) emitted in the direction θ . For the present case of isotropic scattering particles, small as compared with the wave-length of the radiation, $J_\theta = J_0(1 + \cos^2\theta)/2$, where J_0 is the radiant intensity in the direction of the transmitted beam, and the total flux scattered is thus

$$P = \int_0^\pi \frac{J_0}{r^2} \frac{1 + \cos^2\theta}{2} 2\pi r (\sin\theta) r d\theta = \frac{8}{3} \pi J_0 = \frac{16}{3} \pi J_{90}, \quad (9)$$

where J_{90} is the radiant intensity scattered transversely. But from the definition of turbidity,

$$P = P_0 \tau = I_0 V \tau \quad (10)$$

for small turbidities, where P_0 is the flux incident and I_0 the irradiance incident on a volume V , x is the distance traversed in the rectangular volume by the primary beam, and τ is the turbidity (cm.⁻¹). Combining Eqs. (9) and (10), the radiant energy J_{90} scattered transversely is

$$J_{90} = (3/16\pi) I_0 \tau V \quad (11)$$

and the irradiance at a distance r from the scattering center is, omitting refraction and other effects:

$$I = J_{90}/r^2 = (3/16\pi r^2) I_0 \tau V. \quad (12)$$

The actual irradiance I_s at the photo-tube diffusor, after inserting appropriate factors for effects resulting in losses and gains in irradiance, is:

$$I_s = \frac{3(1.045) I_0 \tau (W_s L h) T_s}{16\pi r^2 [1 - (b/r)(n-1)/n]^2 n^2} \quad (13)$$

Included are the refraction factors indicated by Eq. (8); a factor 1.045 to adjust for the effective increase in

TABLE I. Calibration data for turbidity equation.

Quantity	Symbol	436 mμ	546 mμ
Apparent diffuse transmittance of opal glass standard	T	0.315	0.372
Diffusor correction	D	0.840	0.862
Depth of irradiated volume, cm	h	1.20	1.20
Working standard constant	a	0.0159	0.0296
		0.0194 ^a	0.0375 ^a
Neutral filter transmittances	F	See Table III	
Residual refraction correction	R_w/R_c		
Methanol		1.022	1.025
		1.052 ^a	1.067 ^a
Water		1.027	1.029
		1.062 ^a	1.070 ^a
Methyl ethyl ketone		1.054	1.055
		1.067 ^a	1.080 ^a
Benzene		1.067	1.066
		1.099 ^a	1.110 ^a

^a With depolarizing opal glass (O , Figs. 2 and 4) at photo-tube removed.

¹⁷ See also H.-J. Helwig, *Optik* 5, 419 (1949).

TABLE II. Comparison of reflecting diffusors ($i = -45^\circ$, $\alpha = 45^\circ$) with solid opal glass transmitting diffusor (546 m μ).

Diffusor	R	H/V^a	$(G_R/G_0)(T/0.707R)$, Eq. (26)				Diff. between diffusors, %	
			$\frac{1-H/V^b}{1+H/V}$	Obs. ^c	Corr. ^k	Obs. ^l with anal.	Uncorr.	Corr.
Vitrolite ^a	0.816	0.761	0.136	1.33	1.15	1.14	33	15
MgCO ₃ ^b	0.964	0.862	0.074	1.26	1.17	1.16	26	17
MgO ^c	0.99 ^d	0.892	0.057	1.22	1.15	—	22	15
Casein paint No. 1 ^d	0.885	0.934	0.034	1.17	1.13	1.14	17	13
Casein paint No. 2 ^d	0.814	0.939	0.031	1.20	1.16	1.20	20	16
Casein paint ^e powder No. 2	0.871	0.982	0.009	1.19	1.18	1.19	19	18
Flashed opal glass ($T = 0.458$)	—	1.01	—	1.00 ^j	—	—	0	0
Solid opal glass ($T = 0.315$)	—	1.01	—	—	—	—	—	—
Average					1.16	1.17		

^a White structural glass ground with No. 320 carborundum.

^b Block scraped with straightedge.

^c 0.5-mm layer smoked onto MgCO₃ from burning Mg chips.

^d Commercial casein paints of different brands; painted on Vitrolite.

^e Pressed layer of the dry powder, about 1 mm thick on Vitrolite.

^f Absolute reflectance [see F. Benford, G. P. Lloyd, and S. Schwarz, J. Opt. Soc. Am. 38, 445 (1948)]. All other values of R determined on G. E. spectrophotometer relative to MgO and corrected to absolute reflectance.

^g Ratio of deflections for horizontal and vertical setting of analyzer; incident radiation unpolarized.

^h Proportion of reflected radiation polarized [see R. W. Wood, *Physical Optics*, third edition (Macmillan, New York, 1934), p. 341].

ⁱ Analyzer not present when measuring G_R/G_0 .

^j Corrected by assuming $(1-H/V)/(1+H/V)$ is equal to the ratio of specular to diffuse component.

^k Analyzer present (horizontal setting) when determining G_R/G_0 .

^l In this case, the galvanometer deflections for the two types of opal glass are in exact proportion to their observed diffuse transmittances.

incident irradiance due to reflection of a portion of the transmitted beam on emerging from the scattering cell; the reduced volume contributing scattered radiation; and a factor T_s , the transmittance of the filled scattering cell, to account for losses by surface reflections and attenuation by scattering and by solvent absorption.

Referring now to the standard diffusor (Fig. 4), but omitting the compensating cell, the total flux emitted by the viewed irradiated area, A , is obtained by integrating the irradiance I over the surface of a hemisphere of radius r , or by integrating J_α/r^2 over the surface, where J_α is the radiant intensity emitted in a direction α . Assuming that the cosine law of emission holds, $J_\alpha = NA \cos \alpha$, where N is the radiance (erg sec.⁻¹ cm⁻²) of the surface, the total flux emitted is thus

$$P = \int_0^{\pi/2} \frac{NA \cos \alpha}{r^2} 2\pi r (\sin \alpha) r d\alpha = \pi NA. \quad (14)$$

But the total flux emitted is also

$$P = I_0 AT, \quad (15)$$

where T is the diffuse transmittance of the opal glass diffusor. Combining Eqs. (14) and (15) gives $N = I_0 T/\pi$, the radiance of the surface. This value may then be substituted in the cosine law equation $J_\alpha = NA \cos \alpha$, which reduces to

$$J_0 = I_0 AT/\pi \quad (16)$$

for the radiant intensity emitted in the direction of viewing, 0° , for the experimental set-up. The irradiance at a distance r from the diffusor is then

$$I = J_0/r^2 = I_0 AT/\pi r^2. \quad (17)$$

The actual irradiance at the photo-tube diffusor (with the compensating cell in place) is, after inserting

appropriate correction factors:

$$I_a = I_0 (W_s L) T D T_c / \pi r^2 \left(1 - \frac{b}{r} \frac{n-1}{n} \right)^2 \quad (18)$$

In this case the factors are: the refraction factor for foreshortening of r as indicated by Eq. (7); the reduced area contributing radiation to the receiver; a factor T_c , the transmittance of the filled compensating cell, to account for losses in irradiance due to surface reflections and attenuation by scattering and by solvent absorption; and a diffusor correction factor D to adjust for deviation of the standard opal glass from a perfect diffusor (see Section V).

Dividing Eq. (13) by Eq. (18) yields the ratio of irradiances, I_a/I_0 , at the photo-tube diffusor for the scattering solution and the standard diffusor. Solving for turbidity:

$$\tau = \frac{16\pi^2 TD T_c I_s}{3(1.045)h T_s I_0} \quad (19)$$

I_s/I_0 can of course be replaced by FG/G_0 , or the ratio of galvanometer deflections previously discussed (Section III), multiplied by the transmittance F of the neutral filter or filters inserted in the primary beam when reading G_0 . The only geometrical factor remaining in the final equation is h , the depth of the beam. The

Equations (7) to (18) were derived assuming a small source, i.e., that h , W , and L are small compared with r . Integration of Eq. (13), modified by considering a volume element of depth dx , between the limits $x = b - h/2$ and $x = b + h/2$, results in an expression differing from Eq. (13) only in having another second-power term in the parentheses of the denominator which is negligible compared with $2b/r$. Experimental tests involving variation of W , L , and h [see Section VI, tests (1) to (3)] indicate that no significant modification of the practical Eqs. (13), (18), and (19) is required because of the finite size of the volume and area viewed.

ratio of transmittances, T_c/T_s , of the filled cells appears because attenuation is not completely compensated in the substitution method. This ratio has to be determined separately, but not with high accuracy, in a spectrophotometer.

For the more convenient working standard method, an expression analogous to Eq. (18) could be written for the irradiance I_w at the photo-tube diffusor when viewing the working standard at 0° through the solution. However, it is more convenient to derive the turbidity expression for this case empirically from Eq. (19). The working standard can be related to the standard opal glass diffusor by a ratio of deflections in a separate calibration experiment in which the scattering cell and compensating cell are omitted:

$$a = F'G_w'/G_0' = I_w'/I_0'. \quad (20)$$

Here a is the working standard constant, F' the transmittance of an appropriate neutral filter, G_w'/G_0' the ratio of galvanometer deflections, and I_w'/I_0' the ratio of irradiances at the photo-tube diffusor. The ratio of irradiances of Eq. (19) can be written

$$\frac{I_s}{I_0} = \frac{I_s}{I_w} \frac{I_w}{I_0} = \frac{I_s}{I_w} \frac{T_{sa}}{T_{ca}}, \quad (21)$$

where I_w/I_0 is the ratio of irradiances with the filled scattering cell and filled compensating cell in place; T_{sa} is the apparent transmittance of the filled scattering cell, measured in the photometer with the irradiated working standard as a source; and T_{ca} is the apparent transmittance of the filled compensating cell, measured in the photometer with the irradiated standard diffusor as a source. Substituting in Eq. (19),

$$\tau = \frac{16\pi^2 TD}{3(1.045)h} \frac{R_w}{R_c} \frac{I_s}{I_w} \frac{1}{a}, \quad (22)$$

TABLE III. Transmittances of neutral filters (F , photometer; T , spectrophotometer).

Filter No.	Volts per stage	Relative flux level	Measured relative to	F 437 m μ	F 547 m μ	Av. ^b deviation	T 547 m μ	$F-T$ Av.
1	55 115	1 ^a 0.005		0.439	0.508 0.509	0.0008 0.0016	0.506	0.002
2	55 80 59	1 0.06 0.5	No. 1	0.190 0.189	0.257 0.256 0.257	0.0006 0.0011 0.0015	0.257	0.000
3	55 59 65 65	1 0.5 0.25 0.25	No. 1 No. 2 No. 2A	— 0.0988 0.0991	0.155 0.155 0.156	0.0018 0.0006 0.0003	0.156	-0.001
4	65 65 69 72	0.25 0.35 0.15 0.12	No. 2 No. 2A No. 3 No. 4A	0.0761 0.0765 0.0766 0.0760	0.1205 0.1205 0.1206 0.1205	0.0001 0.0004 0.0002 0.0002	0.120	0.000
5	69 72 72	0.15 0.12 0.12	No. 3 No. 4 No. 4A	0.0314 0.0307 0.0312	0.0595 0.0599 0.0595	0.0001 0.0002 0.0003	0.060	0.000

^a With working standard in place (total reduction factor about 2×10^{-4}) and opal glass at photo-tube.

^b Each value of F is a mean calculated from five or six ratios of deflections; the average deviation from the mean is shown for 547 m μ .

where

$$R_w/R_c = (T_{sa}/T_s)/(T_{ca}/T_c). \quad (23)$$

The factor R_w/R_c is seen to be a residual refraction correction made necessary by the fact that the refraction shortening of the distance r is not completely compensated in the working standard method. This ratio can be determined separately for each solvent encountered, and need not be determined for each scattering solution. Experimental values for a number of solvents are shown in Table I. The correction is small and not appreciably dependent on wave-length.

In Eq. (22) the ratio I_s/I_w can be replaced by FG_w/G_0 , the ratio of galvanometer deflections discussed in Section III, multiplied by the transmittance F of the neutral filter or filters** inserted in the primary beam when reading G_w . In the working standard method, reflection losses and attenuation due to scattering and to solvent absorption are completely compensated.

Numerical values for constants of the turbidity equation are assembled in Table I. The diffuse transmittance, T , was determined in a General Electric recording spectrophotometer with the ground surface of the opal glass placed against the entrance aperture of the integrating sphere. Since the plane of the ground surface should be in the plane of the white lining of the sphere, correction factors 1.016 for 436 m μ and 1.025 for 546 m μ were applied to the observed transmittances. These corrections were based on tests with a piece of opal glass cut to fit the entrance hole of the sphere. The transmittances of the neutral filters were determined in the photometer by a stepwise ratio of deflections procedure as indicated in Table III.

V. STANDARD DIFFUSORS

A standard diffusor is advantageous in the evaluation of turbidity, because the diffused primary radiation (as contrasted with the direct parallel primary beam) is similar in its geometry of viewing to that for the diffusely scattered radiation from the solution, thereby simplifying the calculations and permitting at least partial compensation of refraction effects. The diffusor, however, introduces the following difficulties: (1) There are no perfect diffusors; (2) the radiation scattered from reflecting diffusors under the conditions of -45° incidence and 45° viewing was found to be partly polarized, indicating a specular component of reflection; and (3) transmitting diffusors and reflecting diffusors do not yield the same results as standards.

The expression for a perfect reflecting diffusor analogous to Eq. (17), which applies to a perfect transmitting

** An important advantage of the working standard method over the substitution method is that fewer neutral filters (usually one or two) are required in the determination of a turbidity value. This follows from the fact that the intensity from the working standard is intermediate between that from the opal glass standard and that from the scattering solution (see value of constant a in Table I).

diffusor, used under similar conditions, would be

$$I_R = I_0 A R \cos i / \pi r^2, \quad (24)$$

where I_R is the irradiance at a distance r from the diffusing surface of absolute reflectance R , A is the projected irradiated area viewed, i is the angle of incidence, and I_0 the incident irradiance. I_R should be independent of α , the angle of viewing. The ratio of equations (17) and (24) for the condition $i = -45^\circ$ and $\alpha = 45^\circ$ is

$$I/I_R = T/0.707R. \quad (25)$$

This furnishes a means of comparing transmitting and reflecting diffusors as standard diffusing materials, since I/I_R can be measured by a ratio of galvanometer deflections, G_0/G_R , for the opal glass diffusor and a reflecting diffusor placed successively at the table center, with the receiver set at 0° and 90° , respectively, to the primary beam. For diffusors obeying the cosine law of emission,¹⁸ that is, perfect diffusors,

$$(G_R/G_0)(T/0.707R) = 1. \quad (26)$$

Table II gives a comparison, using this relationship, between a number of reflecting diffusors and opal glass transmitting diffusors. It is clear from these data that: (1) With the exception of the casein paint powder, the reflecting diffusors show an appreciable proportion of polarization and hence a specular component in the reflected light, ranging from three percent for casein paint to about 14 percent for Vitrolite. (2) After correction for this effect is made, either by calculation based on determination of percent polarization or by direct measurement (using the analyzer to remove most of the specular component), the reflecting diffusors would yield substantially the same results if used as standards. (3) As a standard transmitting diffusor, flashed opal glass would give the same result as solid opal glass. (4) There is an average discrepancy of 16 percent, however, between the corrected data for the reflecting diffusors and the data for the transmitting diffusors.

Since the ratio indicated by Eq. (26) was found to differ from unity in the comparison of reflecting and transmitting diffusors, it can be concluded that one or both types of diffusors deviate from the cosine law of emission (or that considerable error was made in determining R or T , which is not likely). The literature on such diffusors is not extensive, but indicates that surfaces of magnesium oxide and magnesium carbonate are nearly perfect diffusors under selected conditions. Harrison¹⁹ reported that for magnesium oxide (2 mm thick) the polar reflection curve is the circle required by Lambert's law for the special case of 45° incidence, which was used in the present experiments. Other data²⁰ indicate that magnesium oxide is almost a perfect

TABLE IV. Dependence of multiplier photo-tube response on plane of polarization. Effectiveness of opal glass as depolarizer.

Photo-tube No.	Type	Diffusor at photo-tube	H_u/V_u^*	
			436 m μ	546 m μ
1	931A	None	1.26	1.28
2	931A	None	1.24	1.20
3	931A	None	1.16	1.17
4	931A	None	1.31	1.34
		Flashed opal	1.03	1.05
		Solid opal	1.01	1.02
5	1P21	None	1.03	1.04
		Flashed opal	1.01	1.01
		Solid opal	1.01	1.01
6	1P21	None	1.05	1.04
7	1P21	None	1.04	1.05
8	1P21	None	1.01	1.01

* Ratio of deflections with analyzer set to pass horizontal and vertical electric vector vibrations, respectively, for unpolarized incident radiation, with standard opal glass at table center.

diffusor for conditions of -45° incidence and 0 to 45° viewing. Priest and Riley²¹ observed that a magnesium oxide deposit (~ 0.6 mm) was a less perfect diffusor than common magnesium carbonate block. Hunter,²² in a goniophotometric study of gloss, stated that casein-painted surfaces were the best diffusors he had found. The optical properties of opal glasses have been studied by Ryde and Cooper,²³ and by Luckiesh.²⁴ The latter concluded that flashed and solid opal glasses are both high quality diffusors, but their polar distribution curves and "diffusion coefficients" differ appreciably from those for a perfect diffusor.

It would appear from the foregoing that the diffusing characteristics of magnesium oxide and carbonate surfaces under the conditions of -45° incidence and 45° viewing differ from perfect diffusors only to a minor extent; that these differences are reduced by correction for the small specular component of reflection; and that the corrected reflecting diffusors listed in Table II are superior to opal glass as standards for the present purpose. The opal glass is therefore retained as a secondary or working diffusor having the advantages of permanence, simplicity, and convenience in arranging for compensating certain refraction effects. A diffusor correction is inserted in the turbidity equations (19) and (22), and in Table I, to refer all data to the average of the corrected reflecting diffusors listed in Table II. This correction factor is $D = 1/1.19$ for 436 m μ and $1/1.16$ for 546 m μ .

VI. MISCELLANEOUS TESTS OF PERFORMANCE

Linearity of the photometer and indicating system was tested by comparing the transmittances of the

²¹ I. G. Priest and J. O. Riley, J. Opt. Soc. Am. 20, 156-157A (1930).

²² R. S. Hunter, Paper Trade J. 126, 47 (1948).

²³ J. W. Ryde, Proc. Roy. Soc., London 131A, 451 (1931); J. W. Ryde and B. S. Cooper, *ibid.*, 464; J. W. Ryde and B. S. Cooper, J. Soc. Glass Tech. 16, 408, 430 (1932).

²⁴ M. Luckiesh, Elec. World 60, 1040 (1912); 61, 883 (1913).

¹⁸ A. H. Taylor, Bur. Stand. Sci. Pap. No. 391 (July 28, 1920).

¹⁹ V. G. W. Harrison, Proc. Phys. Soc., London 58, 408 (1946).

²⁰ J. S. Preston, Trans. Opt. Soc., London 31, 15 (1929-30).

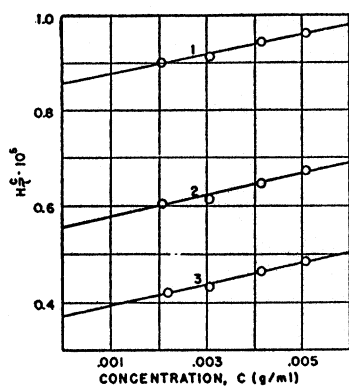


FIG. 6. Plots of Hc/τ versus concentration, for wave-length $436\text{ m}\mu$, for three fractions of polystyrene in methyl ethyl ketone.

neutral filters measured in the photometer at various flux levels with transmittances measured in a recording spectrophotometer at $547\text{ m}\mu$ †† (Table III). The internal consistency of the photometer data and the close agreement with the spectrophotometric data indicate accurate linearity over the range tested. At higher flux levels (e.g., 38 v per stage, relative flux level 16), troublesome photo-tube fatigue effects were encountered. At voltages higher than 115, fluctuations in deflections and dark current were troublesome, particularly under conditions of high relative humidity. Practically all light-scattering measurements were confined to the range of sensitivity settings indicated in Table III. At 115 v per stage, a deflection of 1 mm ($0.001\text{ }\mu\text{A}$) corresponds to a turbidity of about 10^{-6} cm^{-1} .

A number of tests of geometrical factors were made with a test solution of 0.5 percent polystyrene in methyl ethyl ketone. (1) The nose-piece diaphragm (D_4 in Figs. 2 and 4) was replaced by a bilateral slit. The deflection G_s for the scattering solution at 90° was strictly proportional to slit width over the range tested, 1 to 6 mm (adopted width, 3.1 mm). (2) The ratio of galvanometer deflections, G_s/G_0 , in the substitution method was independent of L , the height of the beam over the range tested, 6 to 15 mm (adopted value, 15 mm). (3) Apparent turbidity is to some extent dependent on h , the width of the primary beam, increasing by about two percent when h is changed from 12 (adopted value) to 10 mm, and decreasing by about four percent when h is changed from 12 to 14 mm. This is because the primary beam is not perfectly uniform in cross section. Scanning the 12-mm beam with a movable slit-photo-cell combination showed that the flux was greatest at the center of the beam and that the effective value of h was close to 12 mm for both wave-lengths. (4) Decreasing the divergence of the beam by moving the lamp or adding another lens decreased the apparent turbidity about three percent.

†† Separate transmittance tests in the photometer and spectrophotometer, with selective filters having positive and negative slopes near 436 and $546\text{ m}\mu$, indicated that the continuum for the mercury lamp displaced the effective wave-lengths of the lamp filter combinations by not more than $+1\text{ m}\mu$ from 436 and $546\text{ m}\mu$.

(5) Turbidities determined by the substitution and working standard methods agreed within two percent.

The technique for determination of dissymmetry was tested by observations at 45° and 135° on the fluorescence of fluorescein solutions in water excited by blue light and of erythrosin solutions in alcohol excited by green light. The fluorescence should show no dissymmetry if the concentration is sufficiently low. For the fluorescein solutions, a yellow filter was placed between the solution and the photo-tube to eliminate scattered light, and no correction for solvent was made. For concentrations of 1, 0.5, and 0.1 mg/l, the observed values of G_{45}/G_{135} were 1.012, 1.011, and 1.003, respectively. No filter was used for the erythrosin solutions, but corrections were made for the solvent. Solutions and solvents were filtered by pressure through a fine sintered-glass filter directly into the cleaned dissymmetry cell. For concentrations of 1 and 0.1 mg/l, the observed values were $(99.4-3.2)/(98.4-2.9)=1.007$ and $(77.2-12.8)/(73.4-10.2)=1.019$. The average value G_{45}/G_{135} for all solutions was 1.010, indicating that a correction of -1 percent should be applied to observed dissymmetries for this instrument; however, this is close to the limit of error of a determination.

The scattered light at 90° is in general nearly completely polarized, whereas the light received at 0° is unpolarized. Since the cathode of the multiplier photo-tube is set by construction at about 45° to the incident beam, the response may be dependent on the plane of polarization. The magnitude of this effect differs with different photo-tubes, as shown in Table IV, and unless remedied or corrected, could lead to serious errors with some tubes in the determination of turbidity and depolarization.¹² In the present equipment, in which tubes 3 and 4 were at first used, the effect was remedied by placing a solid opal glass plate near the photo-tube to depolarize the radiation before it strikes the cathode (at the expense, however, of a 10- to 13-fold reduction in intensity). When the effect is large, solid opal glass is superior to flashed opal glass as a depolarizer. When the effect is as small as that shown for the 1P21 tubes, it is advantageous to omit the depolarizing opal plate and apply a small correction in the determination of depolarization; the correction to turbidity in these cases is practically negligible. With the present apparatus, tube 8 is used without the depolarizing diffusor (see Table I for changes in calibration data).

After careful alignment of the Polaroid sheets in the polarizer and analyzer cells with the aid of a Nicol prism at the peephole (H , Fig. 2), the degree of extinction of the crossed Polaroids was determined by ratios of deflections in the photometer at 0° with the aid of neutral filters. The results for $436\text{ m}\mu$ were $H_s/V_s=0.00115$ and $V_h/H_h=0.00116$; for $546\text{ m}\mu$ the corresponding values were 0.00042 and 0.00047. The measurement of small depolarizations should therefore be possible. As a final test, the depolarization of light ($436\text{ m}\mu$) transversely scattered by benzene was meas-

ured. Redistilled benzene was filtered through an ultrafine sintered-glass filter into a carefully cleaned square cell. Correction for stray radiation was made by subtracting deflections observed with the empty cell. An overcorrection might be expected by this procedure, but the final result was actually the same whether the correction was used or omitted, since the stray radiation was only three to five percent of the total and had about the same degree of polarization. Two separate determinations of ρ_u gave 0.458 and 0.456. The average result is nine percent higher than the value 0.420 obtained by Peyrot²⁵ by the Cornu method. The reason for this difference is not known. In a separate experiment with a polystyrene solution, no significant change in the depolarization of 4.5 percent was observed when the angular resolving power of the photometer was increased by narrowing the nose-piece diaphragm (D_4 , Fig. 4) from 3.1 to 1.2 mm.

VII. COMPARISON WITH KNOWN MOLECULAR WEIGHTS AND TURBIDITIES

After careful attention to reducing or eliminating the many sources of error, the final performance of the instrument was tested by determining the molecular weights of polystyrene fractions, a number of proteins, and sucrose octaacetate, the turbidity of a "standard" polystyrene, and Rayleigh's ratio for benzene, and comparing the observed values with results obtained by other methods or other observers.

Polystyrene was prepared and fractionated by a modification of the procedure of Alfrey *et al.*²⁶ Selected fractions having similar viscosity molecular weights were combined and refractionated. Three relatively sharp fractions were finally prepared, and their molecular weights were determined in anhydrous methyl ethyl ketone (MEK) at approximately 25°C by light scattering and by osmotic pressure. For the latter measurements, Zimm²⁷ osmometers with gel Cellophane^{††} membranes were used. Equilibrium was attained in 24 hr. or less. For scattering measurements the solutions

TABLE V. Dissymmetry coefficients, q , depolarization, ρ_u , turbidity correction factors, and refractive index increments.

in μ	Polystyrene fractions (in MEK)						Sucrose octaacetate in methanol	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
q	0.046	0.035	0.065	0.050	0.092	0.061	—	—
Corr. factor ^a	1.033	1.025	1.045	1.035	1.065	1.044	—	—
ρ_u	0.013	0.013	0.015	0.016	0.018	0.017	0.034	—
Corr. factor ^b	0.978	0.978	0.975	0.974	0.970	0.972	0.945	(0.945)
Total corr. ^c	1.009	1.001	1.019	1.008	1.033	1.015	0.945	(0.945)
$(n-n_0)/c$	0.231	0.220	—	—	—	—	0.1165	0.114

^a A randomly coiled model is assumed; see references 2, 3, and 7.

^b $(6-7\rho_u)/(6+3\rho_u)$; see reference 2.

^c Product of turbidity correction factors.

²⁵ P. Peyrot, Comptes Rendus 203, 1512 (1936).

²⁶ T. Alfrey, A. Bartovics, and H. Mark, J. Am. Chem. Soc. 65, 2319 (1943).

²⁷ B. H. Zimm and I. Myerson, J. Am. Chem. Soc. 68, 911 (1946).

^{††} Obtained from Sylvania Corporation, Fredericksburg, Virginia.

TABLE VI. Comparison of light-scattering results with data by other methods or other investigators.

	436 m μ	546 m μ	Average	Other data	Difference, percent
Polystyrene ^a					
Fraction 1	116,000	115,000	115,500	101,000 ^b	14
Fraction 2	180,000	178,000	179,000	151,000 ^b	19
Fraction 3	270,000	268,000	269,000	238,000 ^b	13
Beta-Lactoglobulin ^a	36,000	35,000	35,500	35,500 ^c	0.0
Lysozyme ^a	15,000	14,400	14,700	13,900 ^d	5.8
Bovine serum albumin ^a	72,500	72,500	72,500	69,000 ^e	5.1
Sucrose octaacetate ^a	740	674	709	678 ^f	4.6
Polystyrene, turbidity (cm ⁻¹)	0.00350	0.00131	—	0.00270 ^g	30
				0.00353 ^h	-0.9
				0.00345 ⁱ	1.5
				0.00348 ^j	0.6
				0.00360 ^k	-2.8
Benzene, Rayleigh's ratio $\times 10^6$	48.4	—	—	34.8 ^l	39
				48.4 ^m	0.0
				48.3 ⁿ	0.2
GR-S latex, particle diam. (A)	—	1950	—	2070 ^o	-5.8

^a Molecular weights.

^b By osmotic pressure.

^c By x-ray diffraction, F. R. Senti and R. C. Warner, J. Am. Chem. Soc. 70, 3318 (1948).

^d By x-ray diffraction, K. J. Palmer, M. Ballantyne, and J. A. Galvin, J. Am. Chem. Soc. 70, 906 (1948).

^e By osmotic pressure, G. Scatchard, A. C. Batchelder, and A. Brown, J. Am. Chem. Soc. 68, 2320 (1946).

^f Sum of atomic weights.

^g Dow Styron, kindly supplied in solid form by A. M. Bueche, Cornell University; value quoted is turbidity of 0.5 percent solution in toluene, determined for 436 m μ on the absolute camera (references 2, 4, 5) by J. R. McCartney. A more recent determination on the same camera by E. W. Anacker gave 0.00274.

^h From 90° scattering, 436 m μ , in apparatus similar to ours; P. M. Doty (private communication).

ⁱ By transmission at 436 m μ , P. M. Doty (private communication).

^j Average of two independent methods involving 90° scattering at 436 m μ : 0.00340 and 0.00357; B. H. Zimm (private communication). Other data in reference 6 indicate close agreement between turbidities determined from 90° scattering and from transmission measurements in Zimm's laboratory.

^k From 90° scattering, 436 m μ , F. W. Billmeyer, Jr. (private communication).

^l P. Peyrot, 24°C and 436 m μ , Ann. d. Physik 9, 335 (1938).

^m Reference 6.

ⁿ By electron microscope, number average.

were clarified by pressure filtration through ultrafine sintered-glass filters.

Table V shows data for dissymmetry, depolarization, and refractive index increments for the polystyrene fractions. The data for q and ρ_u apply to a concentration level of 0.004 g/ml. No significant trend in these quantities was detected over the range 0.001 to 0.005 g/ml. Values of $(n-n_0)/c$, determined by means of a differential refractometer,²⁸ were independent of concentration over the range 0.001 to 0.010 g/ml.

Values of Hc/τ versus c are plotted in Fig. 6, in which τ is the excess turbidity of solution over solvent, corrected for dissymmetry and depolarization. Final molecular weight data are assembled in Table VI.

The molecular weights of the polystyrene fractions determined by light scattering (weight-average molecular weights) average 15 percent higher than the osmotic pressure (number-average) values. Very close agreement is not to be expected under these conditions, since the preparation of completely monodisperse fractions is not possible. The difference is in the expected direction.

Table VI also gives results for a number of proteins.

²⁸ B. A. Brice and R. Speiser, J. Opt. Soc. Am. 36, 363A (1946). Complete details will be published.

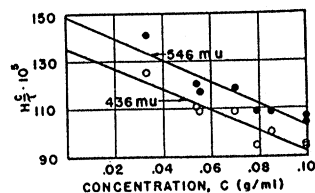


FIG. 7. Plots of Hc/τ versus concentration for wavelengths 436 and 546 $m\mu$ for sucrose octaacetate.

A detailed account of these determinations will be published shortly. The values are in good agreement with those obtained by other methods.

Tables V and VI also give data on sucrose octaacetate, a molecule very small compared with the others listed. The compound (Niacet Company, edible grade) was recrystallized three times from ethyl alcohol. To prepare solutions free of large-particle impurities, solutions in methanol were placed in Cellophane tubes, which were surrounded with redistilled methanol. The solute was allowed to diffuse through the membrane into the outer liquid. After about 24 hr., the outer solution was removed and used for the turbidity measurements, following final cleaning by pressure filtration through ultrafine sintered glass. Concentrations were determined by drying to constant weight at 100°C. The redistilled solvent was treated in the same way for use as a blank. Unless this procedure was followed, results were much too high and very erratic.

The data are plotted in Fig. 7. Each pair of points, one for 436 $m\mu$ and the other for 546 $m\mu$, represents a separate passage through the Cellophane membrane. The best straight lines were drawn through these points by the method of least squares. The average result of 709 is within five percent of the correct molecular weight of 678 and encourages the hope that light scattering may find important application in the intermediate molecular weight range which is too low for measurement by osmotic pressure and by the ultracentrifuge, and too high for accurate measurement by freezing-point depression or vapor-pressure lowering. Determinations of turbidity of sucrose solutions²⁹ and the molecular weight² of sucrose have been published. Reproducibility and accuracy for such substances are not as satisfactory as for materials of high molecular weight because turbidities are small and of the same order of magnitude as the apparent turbidity of the solvent.

Since the light-scattering method is highly sensitive

²⁹ M. Halwer, J. Am. Chem. Soc. 70, 3985 (1948). The coefficient of c^2 in the equation should be 0.02831.

to the presence of aggregates and other large-particle contaminants, high results will in general be obtained unless great care is taken in the clarification of solutions.

Another comparison in Table VI is for the excess turbidity of a 0.5 percent solution of polystyrene in toluene on three different "absolute" instruments. The values shown are 28 to 33 percent higher than the value reported for a solution of the same material determined on the absolute turbidity camera in Debye's laboratory.

The benzene used for determination of Rayleigh's ratio^{§§} was redistilled and filtered into the cleaned scattering cell through an ultrafine sintered-glass filter. Measurements were made by the substitution method. Stray radiation correction was made by subtracting the deflection ratio observed with the empty cell (0.027) from the average deflection ratio with the filled cell (0.930) this may be an overcorrection, but it is not large. The value obtained is considerably higher than that of Peyrot, but in close agreement with the other values cited.

The final comparison is for the particle diameter in a sample of GR-S synthetic rubber latex, as determined by light scattering and by direct measurement with an electron microscope. The light-scattering values were calculated from dissymmetry measurements² on suspensions of four different concentrations in water containing 0.001 percent Duponol. The I_{45}/I_{135} ratio, extrapolated to zero concentration, was 4.34 for wavelength 546 $m\mu$. The agreement between the methods is quite satisfactory.

In conclusion, it would appear that the photometer is capable of determining absolute turbidities, and under ideal conditions weight-average molecular weights, within about ± 5 percent. It is possible, however, that compensating errors are present. The principal uncertainties and sources of error probably concern imperfections of the primary beam and the validity of diffusors as standards.

VIII. ACKNOWLEDGMENTS

The authors are indebted to C. R. Eddy and R. M. Chapman for assistance in the assembly and testing of electrical circuits; to L. P. Witnauer for data on the GR-S latex; and to T. V. Kishbaugh for mechanical work.

^{§§} "Rayleigh's ratio" is the radiant intensity scattered transversely by unit volume as a ratio to the incident irradiance or J_{90}/I_0V [compare with Eq. (11)].